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protofibrils

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- Rosenblum, W. I. (2002). "Structure and location of amyloid beta peptide chains and arrays in Alzheimer's disease: new findings require reevaluation of the amyloid hypothesis and of tests of the hypothesis." Neurobiol Aging **23**(2): 225-30.
New in situ high resolution electronmicroscopic examination of amyloid fibrils in situ indicate that in Alzheimer's disease these fibrils are not simply long chains of self aggregated amyloid beta peptide. The amyloid beta is not only associated with P protein and glycans, as was well known from previous immunohistologic studies, but is arranged in the form of short chains at right angles to a P protein backbone with the glycans wrapped around that backbone. These findings suggest that the hypothesis causally relating simple, fibrillar amyloid beta to Alzheimer's disease must be reevaluated since such simple fibrils may be absent, or not the major form of the amyloid beta in the brain. Other data shows that shorter multimers, so-called protofibrils, or dimers of amyloid beta or molecules cleaved from it can be highly toxic. Some of these may be in the soluble amyloid beta fraction. Shorter multimers or dimers of amyloid beta, either extra or intracellular, may be the real links between amyloid beta production and Alzheimer's disease. Toxicity studies employing fibrillar amyloid beta may not be relevant, even if they produce lesions, because they do not employ amyloid beta in the form in which it actually exists in the Alzheimer brain. Studies of treatments designed to remove fibrils or to prevent their formation may be ineffective or suboptimal in effectiveness because they do not reduce the relevant amyloid burden and/or fail to alter the arrangement of shorter multimers of amyloid beta around its P-protein and glycan core.

Perutz, M. F., J. T. Finch, et al. (2002). "Amyloid fibers are water-filled nanotubes." Proc Natl Acad Sci U S A **99**(8): 5591-5.
A study of papers on amyloid fibers suggested to us that cylindrical beta-sheets are the only structures consistent with some of the x-ray and electron microscope data. We then found that our own 7-year-old and hitherto enigmatic x-ray diagram of

poly-L-glutamine fits a cylindrical sheet of 31 A diameter made of beta-strands with 20 residues per helical turn. Successive turns are linked by hydrogen bonds between both the main chain and side chain amides, and side chains point alternately into and out of the cylinder. Fibers of the exon-1 peptide of huntingtin and of the glutamine- and asparagine-rich region of the yeast prion Sup35 give the same underlying x-ray diagrams, which show that they have the same structure. Electron micrographs show that the 100-A-thick fibers of the Sup35 peptide are ropes made of three protofibrils a little over 30 A thick. They have a measured mass of 1,450 Da/A, compared with 1,426 Da/A for a calculated mass of three protofibrils each with 20 residues per helical turn wound around each other with a helical pitch of 510 A. Published x-ray diagrams and electron micrographs show that fibers of synuclein, the protein that forms the aggregates of Parkinson disease, consist of single cylindrical beta-sheets. Fibers of Alzheimer A beta fragments and variants are probably made of either two or three concentric cylindrical beta-sheets. Our structure of poly-L-glutamine fibers may explain why, in all but one of the neurodegenerative diseases resulting from extension of glutamine repeats, disease occurs when the number of repeats exceeds 37-40. A single helical turn with 20 residues would be unstable, because there is nothing to hold it in place, but two turns with 40 residues are stabilized by the hydrogen bonds between their amides and can act as nuclei for further helical growth. The A beta peptide of Alzheimer's disease contains 42 residues, the best number for nucleating further growth. All these structures are very stable; the best hope for therapies lies in preventing their growth.

Nichols, M. R., M. A. Moss, et al. (2002). "Growth of beta-amyloid(1-40) protofibrils by monomer elongation and lateral association. Characterization of distinct products by light scattering and atomic force microscopy." Biochemistry 41(19): 6115-27.
Amyloid plaques in brain tissue are a hallmark of Alzheimer's disease. Primary components of these plaques are 40- and 42-residue peptides, denoted A beta(1-40) and A beta(1-42), that are derived by proteolysis of cellular amyloid precursor protein. Synthetic A beta(1-40) and A beta(1-42) form amyloid fibrils in vitro that share many features with the amyloid in plaques. Soluble intermediates in A beta fibrillogenesis, termed protofibrils, have been identified previously, and here we describe the in vitro formation and isolation of A beta(1-40) protofibrils by size exclusion chromatography. In some experiments, the A beta(1-40) was radiomethylated to better quantify various A beta species. Mechanistic studies clarified two separate modes of protofibril growth, elongation by monomer deposition and protofibril-protofibril association, that could be resolved by varying the NaCl concentration. Small isolated protofibrils in dilute Tris-HCl buffers were directed along the elongation pathway by addition of A beta(1-40) monomer or along the association pathway by addition of NaCl. Multi-angle light scattering analysis revealed that protofibrils with initial molecular masses M(w) of (7-30) x 10(3) kDa grew to M(w) values of up to 250 x 10(3) kDa by these two growth processes. However, the mass per unit length of the associated protofibrils was about 2-3 times that of the elongated protofibrils. Rate constants for further elongation by monomer deposition with the elongated, associated, and initial protofibril pools were identical when equal number concentrations of original protofibrils were compared,

indicating that the original number of protofibril ends had not been altered by the elongation or association processes. Atomic force microscopy revealed heterogeneous initial protofibrils that became more rodlike following the elongation reaction. Our data indicate that protofibril elongation in the absence of NaCl results from monomer deposition only at the ends of protofibrils and proceeds without an increase in protofibril diameter. In contrast, protofibril association occurs in the absence of monomer when NaCl is introduced, but this association involves lateral interactions that result in a relatively disordered fibril structure.

Lashuel, H. A., D. Hartley, et al. (2002). "Neurodegenerative disease: amyloid pores from pathogenic mutations." Nature **418**(6895): 291.
Alzheimer's and Parkinson's diseases are associated with the formation in the brain of amyloid fibrils from beta-amyloid and alpha-synuclein proteins, respectively. It is likely that oligomeric fibrillization intermediates (protofibrils), rather than the fibrils themselves, are pathogenic, but the mechanism by which they cause neuronal death remains a mystery. We show here that mutant amyloid proteins associated with familial Alzheimer's and Parkinson's diseases form morphologically indistinguishable annular protofibrils that resemble a class of pore-forming bacterial toxins, suggesting that inappropriate membrane permeabilization might be the cause of cell dysfunction and even cell death in amyloid diseases.

Klein, W. L. (2002). "ADDLs & protofibrils--the missing links?" Neurobiol Aging **23**(2): 231-5.

Klein, W. (2002). "Abeta toxicity in Alzheimer's disease: globular oligomers (ADDLs) as new vaccine and drug targets." Neurochem Int **41**(5): 345.
Over the past several years, experiments with synthetic amyloid-beta peptide (Abeta) and animal models have strongly suggested that pathogenesis of Alzheimer's disease (AD) involves soluble assemblies of Abeta peptides (Trends Neurosci. 24 (2001) 219). These soluble neurotoxins (known as ADDLs and protofibrils) seem likely to account for the imperfect correlation between insoluble fibrillar amyloid deposits and AD progression. Recent experiments have detected the presence of ADDLs in AD-afflicted brain tissue and in transgenic-mice models of AD. The presence of high affinity ADDL binding proteins in hippocampus and frontal cortex but not cerebellum parallels the regional specificity of AD pathology and suggests involvement of a toxin receptor-mediated mechanism. The properties of ADDLs and their presence in AD-afflicted brain are consistent with their putative role even in the earliest stages of AD, including forms of mild cognitive impairment.

Behl, C. and B. Moosmann (2002). "Oxidative nerve cell death in Alzheimer's disease and stroke: antioxidants as neuroprotective compounds." Biol Chem **383**(3-4): 521-36.
Many neurodegenerative disorders and syndromes are associated with an excessive generation of reactive oxygen species (ROS) and oxidative stress. The pathways to nerve cell death induced by diverse potential neurotoxins such as peptides, excitatory amino acids, cytokines or synthetic drugs commonly share oxidative downstream

processes, which can cause either an acute oxidative destruction or activate secondary events leading to apoptosis. The pathophysiological role of ROS has been intensively studied in in vitro and in vivo models of chronic neurodegenerative diseases such as Alzheimer's disease (AD) and of syndromes associated with rapid nerve cell loss as occuring in stroke. In AD, oxidative neuronal cell dysfunction and cell death caused by protofibrils and aggregates of the AD-associated amyloid beta protein (Abeta) may causally contribute to pathogenesis and progression. ROS and reactive nitrogen species also take part in the complex cascade of events and the detrimental effects occuring during ischemia and reperfusion in stroke. Direct antioxidants such as chain-breaking free radical scavengers can prevent oxidative nerve cell death. Although there is ample experimental evidence demonstrating neuroprotective activities of direct antioxidants in vitro, the clinical evidence for antioxidant compounds to act as protective drugs is relatively scarce. Here, the neuroprotective potential of antioxidant phenolic structures including alpha-tocopherol (vitamin E) and 17beta-estradiol (estrogen) in vitro is summarized. In addition, the antioxidant and cytoprotective activities of lipophilic tyrosine- and tryptophan-containing structures are discussed. Finally, an outlook is given on the neuroprotective potential of aromatic amines and imines, which may comprise novel lead structures for antioxidant drug design.

Nilsberth, C., A. Westlind-Danielsson, et al. (2001). "The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Abeta protofibril formation." Nat Neurosci 4(9): 887-93.
Several pathogenic Alzheimer's disease (AD) mutations have been described, all of which cause increased amyloid beta-protein (Abeta) levels. Here we present studies of a pathogenic amyloid precursor protein (APP) mutation, located within the Abeta sequence at codon 693 (E693G), that causes AD in a Swedish family. Carriers of this 'Arctic' mutation showed decreased Abeta42 and Abeta40 levels in plasma. Additionally, low levels of Abeta42 were detected in conditioned media from cells transfected with APPE693G. Fibrillization studies demonstrated no difference in fibrillization rate, but Abeta with the Arctic mutation formed protofibrils at a much higher rate and in larger quantities than wild-type (wt) Abeta. The finding of increased protofibril formation and decreased Abeta plasma levels in the Arctic AD may reflect an alternative pathogenic mechanism for AD involving rapid Abeta protofibril formation leading to accelerated buildup of insoluble Abeta intra- and/or extracellularly.

Lambert, M. P., K. L. Viola, et al. (2001). "Vaccination with soluble Abeta oligomers generates toxicity-neutralizing antibodies." J Neurochem 79(3): 595-605.
In recent studies of transgenic models of Alzheimer's disease (AD), it has been reported that antibodies to aged beta amyloid peptide 1-42 (Abeta(1-42)) solutions (mixtures of Abeta monomers, oligomers and amyloid fibrils) cause conspicuous reduction of amyloid plaques and neurological improvement. In some cases, however, neurological improvement has been independent of obvious plaque reduction, and it has been suggested that immunization might neutralize soluble, non-fibrillar forms of Abeta. It is now known that Abeta toxicity resides not only in fibrils, but also in soluble protofibrils and oligomers. The current study has investigated the immune response to

low doses of Abeta(1-42) oligomers and the characteristics of the antibodies they induce. Rabbits that were injected with Abeta(1-42) solutions containing only monomers and oligomers produced antibodies that preferentially bound to assembled forms of Abeta in immunoblots and in physiological solutions. The antibodies have proven useful for assays that can detect inhibitors of oligomer formation, for immunofluorescence localization of cell-attached oligomers to receptor-like puncta, and for immunoblots that show the presence of SDS-stable oligomers in Alzheimer's brain tissue. The antibodies, moreover, were found to neutralize the toxicity of soluble oligomers in cell culture. Results support the hypothesis that immunizations of transgenic mice derive therapeutic benefit from the immuno-neutralization of soluble Abeta-derived toxins. Analogous immuno-neutralization of oligomers in humans may be a key in AD vaccines.

Klein, W. L., G. A. Krafft, et al. (2001). "Targeting small Abeta oligomers: the solution to an Alzheimer's disease conundrum?" Trends Neurosci **24**(4): 219-24.
Amyloid beta (Abeta) is a small self-aggregating peptide produced at low levels by normal brain metabolism. In Alzheimer's disease (AD), self-aggregation of Abeta becomes rampant, manifested most strikingly as the amyloid fibrils of senile plaques. Because fibrils can kill neurons in culture, it has been argued that fibrils initiate the neurodegenerative cascades of AD. An emerging and different view, however, is that fibrils are not the only toxic form of Abeta, and perhaps not the neurotoxin that is most relevant to AD: small oligomers and protofibrils also have potent neurological activity. Immuno-neutralization of soluble Abeta-derived toxins might be the key to optimizing AD vaccines that are now on the horizon.

Haass, C. and H. Steiner (2001). "Protofibrils, the unifying toxic molecule of neurodegenerative disorders?" Nat Neurosci **4**(9): 859-60.

Gregoire, C., S. Marco, et al. (2001). "Three-dimensional structure of the lithostathine protofibril, a protein involved in Alzheimer's disease." Embo J **20**(13): 3313-21.
Neurodegenerative diseases are characterized by the presence of filamentous aggregates of proteins. We previously established that lithostathine is a protein overexpressed in the pre-clinical stages of Alzheimer's disease. Furthermore, it is present in the pathognomonic lesions associated with Alzheimer's disease. After self-proteolysis, the N-terminally truncated form of lithostathine leads to the formation of fibrillar aggregates. Here we observed using atomic force microscopy that these aggregates consisted of a network of protofibrils, each of which had a twisted appearance. Electron microscopy and image analysis showed that this twisted protofibril has a quadruple helical structure. Three-dimensional X-ray structural data and the results of biochemical experiments showed that when forming a protofibril, lithostathine was first assembled via lateral hydrophobic interactions into a tetramer. Each tetramer then linked up with another tetramer as the result of longitudinal electrostatic interactions. All these results were used to build a structural model for the lithostathine protofibril called the quadruple-helical filament (QHF-litho). In conclusion, lithostathine strongly resembles the prion protein in its dramatic

proteolysis and amyloid proteins in its ability to form fibrils.

El-Agnaf, O. M., S. Nagala, et al. (2001). "Non-fibrillar oligomeric species of the amyloid ABri peptide, implicated in familial British dementia, are more potent at inducing apoptotic cell death than protofibrils or mature fibrils." J Mol Biol **310**(1): 157-68.
Familial British dementia (FBD) is an autosomal dominant neurodegenerative disorder, with biochemical and pathological similarities to Alzheimer's disease. FBD is associated with a point mutation in the stop codon of the BRI gene. The mutation extends the length of the wild-type protein by 11 amino acids, and following proteolytic cleavage, results in the production of a cyclic peptide (ABri) 11 amino acids longer than the wild-type (WT) peptide produced from the normal gene BRI. ABri was found to be the main component of amyloid deposits in FBD brains. However, pathological examination of FBD brains has shown the presence of ABri as non-fibrillar deposits as well as amyloid fibrils. Taken together, the genetic, pathological and biochemical data support the hypothesis that ABri deposits play a central role in the pathogenesis of FBD. Here we report that ABri, but not WT peptide, can oligomerise and form amyloid-like fibrils. We show for the first time that ABri induces apoptotic cell death, whereas WT is not toxic to cells. Moreover, we report the novel findings that non-fibrillar oligomeric species of ABri are more toxic than protofibrils and mature fibrils. These findings provide evidence that non-fibrillar oligomeric species are likely to play a critical role in the pathogenesis of FBD and suggest that a similar process may also operate in other neurodegenerative diseases.

Dumery, L., F. Bourdel, et al. (2001). "beta-Amyloid protein aggregation: its implication in the physiopathology of Alzheimer's disease." Pathol Biol (Paris) **49**(1): 72-85.
beta-Amyloid protein (A beta), a 39-42 residue peptide resulting from the proteolytic processing of a membrane-bound beta-amyloid precursor protein (APP), is one of the major components of the fibrillar deposits observed in Alzheimer patients. A beta fibril formation is a complex process which involves changes in A beta conformation and self-association to form cross-beta pleated sheets, protofibrils, and fibrils. Since the aggregation of soluble A beta peptide into fibrils is viewed as a critical event in the physiopathology of Alzheimer's disease (AD), preventing, altering, or reversing fibril formation may thus be of therapeutic value. This review will focus on the current state of knowledge of A beta fibril formation, with special emphasis on physiological and exogenous inhibitors which may have a therapeutic potential.

Ward, R. V., K. H. Jennings, et al. (2000). "Fractionation and characterization of oligomeric, protofibrillar and fibrillar forms of beta-amyloid peptide." Biochem J **348 Pt 1**: 137-44.
The beta-amyloid (Abeta) peptide, a major component of senile plaques in Alzheimer's disease brain, has been shown previously to undergo a process of polymerization to produce neurotoxic forms of amyloid. Recent literature has attempted to define precisely the form of Abeta responsible for its neurodegenerative properties. In the

present study we describe a novel density-gradient centrifugation method for the isolation and characterization of structurally distinct polymerized forms of Abeta peptide. Fractions containing protofibrils, fibrils, sheet structures and low molecular mass oligomers were prepared. The fractionated forms of Abeta were characterized structurally by transmission electron microscopy. The effects on cell viability of these fractions was determined in the B12 neuronal cell line and hippocampal neurons. Marked effects on cell viability in the cells were found to correspond to the presence of protofibrillar and fibrillar structures, but not to monomeric peptide or sheet-like structures of polymerized Abeta. Biological activity correlated with a positive reaction in an immunoassay that specifically detects protofibrillar and fibrillar Abeta; those fractions that were immunoassay negative had no effect on cell viability. These data suggest that the effect of Abeta on cell viability is not confined to a single conformational form but that both fibrillar and protofibrillar species have the potential to be active in this assay.

Stolz, M., D. Stoffler, et al. (2000). "Monitoring biomolecular interactions by time-lapse atomic force microscopy." J Struct Biol **131**(3): 171-80.
The atomic force microscope (AFM) is a unique imaging tool that enables the tracking of single macromolecule events in response to physiological effectors and pharmacological stimuli. Direct correlation can therefore be made between structural and functional states of individual biomolecules in an aqueous environment. This review explores how time-lapse AFM has been used to learn more about normal and disease-associated biological processes. Three specific examples have been chosen to illustrate the capabilities of this technique. In the cell, actin polymerizes into filaments, depolymerizes, and undergoes interactions with numerous effector molecules (i.e., severing, capping, depolymerizing, bundling, and cross-linking proteins) in response to many different stimuli. Such events are critical for the function and maintenance of the molecular machinery of muscle contraction and the dynamic organization of the cytoskeleton. One goal is to use time-lapse AFM to examine and manipulate some of these events in vitro, in order to learn more about how these processes occur in the cell. Aberrant protein polymerization into amyloid fibrils occurs in a multitude of diseases, including Alzheimer's and type 2 diabetes. Local amyloid deposits may cause organ dysfunction and cell death; hence, it is of interest to learn how to interfere with fibril formation. One application of time-lapse AFM in this area has been the direct visualization of amyloid fibril growth in vitro. This experimental approach holds promise for the future testing of potential therapeutic drugs, for example, by directly visualizing at which level of fibril assembly (i.e., nucleation, elongation, branching, or lateral association of protofibrils) a given active compound will interfere. Nuclear pore complexes (NPCs) are large supramolecular assemblies embedded in the nuclear envelope. Transport of ions, small molecules, proteins, RNAs, and RNP particles in and out of the nucleus occurs via NPCs. Time-lapse AFM has been used to structurally visualize the response of individual NPC particles to various chemical and physical effectors known to interfere with nucleocytoplasmic transport. Taken together, such time-lapse AFM studies could provide novel insights into the molecular mechanisms of fundamental biological processes under both normal and

pathological conditions at the single molecule level.

Serpell, L. C. (2000). "Alzheimer's amyloid fibrils: structure and assembly." Biochim Biophys Acta **1502**(1): 16-30.
Structural studies of Alzheimer's amyloid fibrils have revealed information about the structure at different levels. The amyloid-beta peptide has been examined in various solvents and conditions and this has led to a model by which a conformational switching occurs from alpha-helix or random coil, to a beta-sheet structure. Amyloid fibril assembly proceeds by a nucleation dependent pathway leading to elongation of the fibrils. Along this pathway small oligomeric intermediates and short fibrillar structures (protofibrils) have been observed. In cross-section the fibril appears to be composed of several subfibrils or protofilaments. Each of these protofilaments is composed of beta-sheet structure in which hydrogen bonding occurs along the length of the fibre and the beta-strands run perpendicular to the fibre axis. This hierarchy of structure is discussed in this review.

McLaurin, J., D. Yang, et al. (2000). "Review: modulating factors in amyloid-beta fibril formation." J Struct Biol **130**(2-3): 259-70.
Amyloid formation is a key pathological feature of Alzheimer's disease and is considered to be a major contributing factor to neurodegeneration and clinical dementia. Amyloid is found as both diffuse and senile plaques in the parenchyma of the brain and is composed primarily of the 40- to 42-residue amyloid-beta (Abeta) peptides. The characteristic amyloid fiber exhibits a high beta-sheet content and may be generated in vitro by the nucleation-dependent self-association of the Abeta peptide and an associated conformational transition from random to beta-conformation. Growth of the fibrils occurs by assembly of the Abeta seeds into intermediate protofibrils, which in turn self-associate to form mature fibers. This multistep process may be influenced at various stages by factors that either promote or inhibit Abeta fiber formation and aggregation. Identification of these factors and understanding the driving forces behind these interactions as well as the structural motifs necessary for these interactions will help to elucidate potential sites that may be targeted to prevent amyloid formation and its associated toxicity. This review will discuss some of the modulating factors that have been identified to date and their role in fibrillogenesis.

Huang, T. H., D. S. Yang, et al. (2000). "Structural studies of soluble oligomers of the Alzheimer beta-amyloid peptide." J Mol Biol **297**(1): 73-87.
Recent studies have suggested that non-fibrillar soluble forms of Abeta peptides possess neurotoxic properties and may therefore play a role in the molecular pathogenesis of Alzheimer's disease. We have identified solution conditions under which two types of soluble oligomers of Abeta40 could be trapped and stabilized for an extended period of time. The first type of oligomers comprises a mixture of dimers/tetramers which are stable at neutral pH and low micromolar concentration, for a period of at least four weeks. The second type of oligomer comprises a narrow distribution of particles that are spherical when examined by electron microscopy and

atomic force microscopy. The number average molecular mass of this distribution of particles is 0.94 MDa, and they are are stable at pH 3 for at least four weeks. Circular dichroism studies indicate that the dimers/tetramers possess irregular secondary structure that is not alpha-helix or beta-structure, while the 0.94 MDa particles contain beta-structure. Fluorescence resonance energy transfer experiments indicate that Abeta40 moieties in amyloid fibrils or protofibrils are more similar in structure to those in the 0.94 MDa particles than those in the dimers/tetramers. These findings indicate that soluble oligomeric forms of Abeta peptides can be trapped for extended periods of time, enabling their study by high resolution techniques that would not otherwise be possible.

Goldsbury, C. S., S. Wirtz, et al. (2000). "Studies on the in vitro assembly of a beta 1-40: implications for the search for a beta fibril formation inhibitors." J Struct Biol **130**(2-3): 217-31.
The progressive deposition of the amyloid beta peptide (Abeta) in fibrillar form is a key feature in the development of the pathology in Alzheimer's disease (AD). We have characterized the time course of Abeta fibril formation using a variety of assays and under different experimental conditions. We describe in detail the morphological development of the Abeta polymerization process from pseudo-spherical structures and protofibrils to mature thioflavin-T-positive/Congo red-positive amyloid fibrils. Moreover, we structurally characterize the various polymorphic fibrillar assemblies using transmission electron microscopy and determine their mass using scanning transmission electron microscopy. These results provide the framework for future investigations into how target compounds may interfere with the polymerization process. Such substances might have a therapeutic potential in AD.

Yang, D. S., C. M. Yip, et al. (1999). "Manipulating the amyloid-beta aggregation pathway with chemical chaperones." J Biol Chem **274**(46): 32970-4.
Amyloid-beta (Abeta) assembly into fibrillar structures is a defining characteristic of Alzheimer's disease that is initiated by a conformational transition from random coil to beta-sheet and a nucleation-dependent aggregation process. We have investigated the role of organic osmolytes as chemical chaperones in the amyloid pathway using glycerol to mimic the effects of naturally occurring molecules. Osmolytes such as the naturally occurring trimethylamine N-oxide and glycerol correct folding defects by preferentially hydrating partially denatured proteins and entropically stabilize native conformations and polymeric states. Trimethylamine N-oxide and glycerol were found to rapidly accelerate the Abeta random coil-to-beta-sheet conformational change necessary for fiber formation. This was accompanied by an immediate conversion of amorphous unstructured aggregates into uniform globular and possibly nucleating structures. Osmolyte-facilitated changes in Abeta hydration also affected the final stages of amyloid formation and mediated transition from the protofibrils to mature fibers that are observed in vivo. These findings suggest that hydration forces can be used to control fibril assembly and may have implications for the accumulation of Abeta within intracellular compartments such as the endoplasmic reticulum and in vitro modeling of the amyloid pathway.

Walsh, D. M., D. M. Hartley, et al. (1999). "Amyloid beta-protein fibrillogenesis. Structure and biological activity of protofibrillar intermediates." J Biol Chem **274**(36): 25945-52.
Alzheimer's disease is characterized by extensive cerebral amyloid deposition. Amyloid deposits associated with damaged neuropil and blood vessels contain abundant fibrils formed by the amyloid beta-protein (Abeta). Fibrils, both in vitro and in vivo, are neurotoxic. For this reason, substantial effort has been expended to develop therapeutic approaches to control Abeta production and amyloidogenesis. Achievement of the latter goal is facilitated by a rigorous mechanistic understanding of the fibrillogenesis process. Recently, we discovered a novel intermediate in the pathway of Abeta fibril formation, the amyloid protofibril (Walsh, D. M., Lomakin, A., Benedek, G. B., Condron, M. M., and Teplow, D. B. (1997) J. Biol. Chem. 272, 22364-22372). We report here results of studies of the assembly, structure, and biological activity of these polymers. We find that protofibrils: 1) are in equilibrium with low molecular weight Abeta (monomeric or dimeric); 2) have a secondary structure characteristic of amyloid fibrils; 3) appear as beaded chains in rotary shadowed preparations examined electron microscopically; 4) give rise to mature amyloid-like fibrils; and 5) affect the normal metabolism of cultured neurons. The implications of these results for the development of therapies for Alzheimer's disease and for our understanding of fibril assembly are discussed.

Hartley, D. M., D. M. Walsh, et al. (1999). "Protofibrillar intermediates of amyloid beta-protein induce acute electrophysiological changes and progressive neurotoxicity in cortical neurons." J Neurosci **19**(20): 8876-84.
Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is thought to be caused in part by the age-related accumulation of amyloid beta-protein (Abeta). The presence of neuritic plaques containing abundant Abeta-derived amyloid fibrils in AD brain tissue supports the concept that fibril accumulation per se underlies neuronal dysfunction in AD. Recent observations have begun to challenge this assumption by suggesting that earlier Abeta assemblies formed during the process of fibrillogenesis may also play a role in AD pathogenesis. Here, we present the novel finding that protofibrils (PF), metastable intermediates in amyloid fibril formation, can alter the electrical activity of neurons and cause neuronal loss. Both low molecular weight Abeta (LMW Abeta) and PF reproducibly induced toxicity in mixed brain cultures in a time- and concentration-dependent manner. No increase in fibril formation during the course of the experiments was observed by either Congo red binding or electron microscopy, suggesting that the neurotoxicity of LMW Abeta and PF cannot be explained by conversion to fibrils. Importantly, protofibrils, but not LMW Abeta, produced a rapid increase in EPSPs, action potentials, and membrane depolarizations. These data suggest that PF have inherent biological activity similar to that of mature fibrils. Our results raise the possibility that the preclinical and early clinical progression of AD is driven in part by the accumulation of specific Abeta assembly intermediates formed during the process of fibrillogenesis.

Harper, J. D., S. S. Wong, et al. (1999). "Assembly of A beta amyloid protofibrils: an in vitro model for a possible early event in Alzheimer's disease." Biochemistry 38(28): 8972-80.
Amyloid fibrils comprising primarily the peptides A beta 40 and A beta 42 are a defining feature of the Alzheimer's disease (AD) brain, and convergent evidence suggests that the process of their formation plays a central role in the AD pathogenic pathway. Elucidation of fibril assembly is critical for the discovery of potential AD diagnostics and therapeutics, since the pathogenic entity is not necessarily the product fibril, but could be a precursor species whose formation is linked to fibrillogenesis in vivo. Atomic force microscopy allowed the identification of an unanticipated intermediate in in vitro fibril formation, the A beta amyloid protofibril. This manuscript describes studies of the structure of the A beta 40 protofibril and its in vitro assembly and disassembly using atomic force microscopy (AFM). The A beta 40 protofibril has a height of ca. 4.3 +/- 0.5 nm and a periodicity of ca. 20 +/- 4.7 nm. The rate of its elongation depends on the total concentration of A beta 40, the temperature, and ionic strength of the medium. A beta 42 and A beta 40 protofibrils elongate at a comparable rate. Statistical analysis of AFM data reveals a decrease in the number of protofibrils with time, indicating that coalescence of smaller protofibrils contributes to protofibril elongation. Similar analysis reveals that protofibrils shorten while the number of protofibrils also decrease following dilution, indicating that protofibril disassembly does not proceed by a reverse of the assembly process. These investigations provide systematic data defining factors affecting A beta fibrillization and, thus, should be valuable in the design of high-throughput assays to identify agents which alter A beta protofibril assembly.

Harper, J. D., C. M. Lieber, et al. (1997). "Atomic force microscopic imaging of seeded fibril formation and fibril branching by the Alzheimer's disease amyloid-beta protein." Chem Biol 4(12): 951-9.
BACKGROUND: Amyloid plaques composed of the fibrillar form of the amyloid-beta protein (Abeta) are the defining neuropathological feature of Alzheimer's disease (AD). A detailed understanding of the time course of amyloid formation could define steps in disease progression and provide targets for therapeutic intervention. Amyloid fibrils, indistinguishable from those derived from an AD brain, can be produced in vitro using a seeded polymerization mechanism. In its simplest form, this mechanism involves a cooperative transition from monomeric Abeta to the amyloid fibril without the buildup of intermediates. Recently, however, a transient species, the Abeta amyloid protofibril, has been identified. Here, we report studies of Abeta amyloid protofibril and its seeded transition into amyloid fibrils using atomic force microscopy. RESULTS: Seeding of the protofibril-to-fibril transition was observed. Preformed fibrils, but not protofibrils, effectively seeded this transition. The assembly state of Abeta influenced the rate of seeded growth, indicating that protofibrils are fibril assembly precursors. The handedness of the helical surface morphology of fibrils depended on the chirality of Abeta. Finally, branched and partially wound fibrils were observed. CONCLUSIONS: The temporal evolution of morphologies suggests that the protofibril-to-fibril transition is nucleation-dependent and that protofibril winding is involved in that transition. Fibril

unwinding and branching may be essential for the post-nucleation growth process. The protofibrillar assembly intermediate is a potential target for AD therapeutics aimed at inhibiting amyloid formation and AD diagnostics aimed at detecting presymptomatic disease.